Amendments to the Drawings:

The attached sheets of drawings include changes to Figs. 2:

Sheet 2, which includes Fig. 2, replaces the sheet that includes Fig. 2.

Attachment: 1 replacement sheet(s).

REMARKS

Claims 1-19 are currently pending in the application. Claims 1, 4, 7, 12, 13, 14 and 19 are amended. The amendments find support in the specification and are discussed in the relevant sections below. No new matter is added.

Specification

1. The disclosure is objected to because on page 33, the specification refers to SEQ ID NO:1 on Figure 6. However, there are only five figures in the disclosure.

Applicant has amended the specification so that the phrase "Figure 6" is deleted from the above referenced excerpt of the specification.

2. The disclosure is objected to because of the trademark STRATAPREP is not capitalized, accompanied by generic terminology.

Applicant has amended the specification on page 32 so that the above referenced trademark is capitalized and is accompanied by generic terminology.

3A. The specification is objected to as failing to provide proper antecedent basis for the claimed subject matter of claims 4, 12, 13 and 19 which states "wherein in one or both of said first and second vectors there is no second site specific recombinase recognition site between said double-stranded origin of replication and said-site specific recombinase recognition site", but it is not disclosed in the remainder of the specification

3B. The specification is also objected to as failing to provide proper antecedent basis for the claimed subject matter of claim 10, in which the *strA* gene is listed as a selectable marker but it is not disclosed in the remainder of the specification.

As noted in the office action, the claims as filed in the original application are part of the disclosure and therefore, the applicant may amend the specification to include the claimed subject matter without adding new matter.

Accordingly Applicant has amended the specification to contain the above referenced subject matter of claims 4, 12, 13, 19 and 10.

Claim Objections

1. Claim 1 is objected to because claim 1 recites the phrase "initiate replication "as" said double stranded" wherein the word "as" appears to be a typo.

Applicant has amended claim 1 so that the referenced word "as" in the phrase "initiate replication "as" said double stranded" is replaced by the word "at".

2. Claim 14 is objected to for having a double period at the end of the claim.

Applicant has amended the claim to remove the second period.

Claim Rejections – 35 U.S.C. § 112

1. Claims 1, 4, 9, 12-13 and 19 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite because it is not clear in what way the second vector comprises a negative selectable marker.

In order to more particularly and distinctly point out the invention, Applicant has amended claims 1, 4, 12-13 and 19 to recite "*a gene* encoding a negative selectable marker", (emphasis added). Applicant contends that this amendment to claims 1, 4, 12-13 and 19, renders claims 1, 4, 12-13 and 19, and claim 9 which is dependent on claim 4, definite, and respectfully request withdrawal of the rejection.

2. The Office Action states that claim 6 is rejected as being vague and indefinite because it recites a limitation of claim 4 "wherein said site-specific recombinase recognition site is selected from the group consisting of ...", (emphasis added), and there are multiple site-specific recombination sites listed in claim 4. The Office Action further states that it is not clear whether each of the site specific recombinase recognition sites in the first and second vectors of claim 4 must have the same recognition sites or whether they may have site-specific recombinase recognition sites that are different from each other.

In order to more particularly define the invention, Applicant has amended the claims to indicate that the site specific recombinase recognition sites in the first and second vectors of claim 4 are recognized by the same site specific recombinase. As disclosed in the specification on page 23, the two lox sites which differ due to variations in their binding sites may be recombined provided that the enzyme can bind to each of the variant binding sites of the Cre recombinase enzyme. Therefore, no new matter is added.

3. The Office Action states that Claim 7 is rejected as being vague and indefinite because "it recites a limitation of claim 4 identifying the double stranded origin of replication as being from the filamentous bacteriophage f1" (emphasis added). The office action contends that since the first and second vector each have a double stranded origin of replication, it is not clear whether each of the double-stranded origins of replication in the first and second vectors of claim 4 must be both from the filamentous bacteriphage f1 or whether one may be from a different source.

In order to more particularly define the invention, Applicant has amended claim 7 to indicate that the double-stranded origin of replications in both the first and second vectors of claim 4 are the double-stranded origin of replication of the filamentous bacteriophage f1. Support for this amendment is found on page 33, lines 23-29. No new matter is added.

4. The Office Action states that Claim 8 is rejected as being vague and indefinite "because it recites a limitation of claim 4 identifying the double stranded origin of replication as being from the plasmid pKym" (emphasis added). The Office action further states that since the first and second vector each have a double stranded origin of replication, it is not clear whether each of the double-stranded origin of replications in the first and second vectors of claim 4 must be both from the plasmid pKym or whether one may be from a different source.

In order to more particularly define the invention, Applicant has amended the claims to indicate that the double stranded origin of replications in both the first and second vectors of claim 4 are from the plasmid pKym. Support for this amendment is found on page 33, lines 23-29. No new matter is added.

5. The Office Action states that Claim 1 is rejected as being vague and indefinite because the metes and bounds of the limitation "interposed between" are not clear in the phrase "gene of interest interposed between said double-stranded origin of replication of said second vector and said site specific recombination recognition site, said single stranded origin of replication of said second vector, and said gene encoding said second selectable marker". The Office Action further states that the definition of an "interposed" gene of interest is disclosed in the specification (pages 7-8) as being :

> " a nucleic acid molecule which has, immediately adjacent to its 5' most end, either a double-stranded origin of replication of a rolling circle replicon or a site specific recombination recognition site, and has immediately adjacent to its 3' most end which ever of the double stranded origin of replication of a rolling circle replicon or site specific recombination recognition site is that is not immediately adjacent to the 5' most end".

The office action states that it is clear from this definition that the gene of interest is interposed between two elements which are immediately adjacent on either side of the gene of interest. The Office Action further states that as claim 1 is written, it is apparent that the gene of interest is adjacent, on one side, to the double stranded origin of replication of said vector; however, it is not clear which of the next three listed elements (i.e. the site specific recombination recognition site, the single stranded origin of replication of said second vector, and the gene encoding said second selectable marker) is on the other side of the gene of interest, so that the gene of interest would be "interposed between" the elements.

Applicant has amended claim 1 to clarify that the gene of interest is interposed between the double stranded origin of replication of said second vector, and the site specific recombination recognition site.

6. Claim 1 is vague and indefinite because it does not establish a functional link between the introduction of a Rep protein which initiates replication and formation of the product vector. The office action contends that it is not clear from the claim language how introduction of a Rep protein and the initiation of replication produce a product vector.

Applicant has amended claim 1 to clarify that the presence of a Rep protein in the host cell functions to initiate ***rolling circle*** replication ***at*** the double stranded origins of replication by

substituting "at" for "as" following the phrase "wherein said host cell further expresses a gene encoding a Rep protein that can initiate replication", and by inserting "rolling circle" into the phrase immediately preceding the word "replication".

The present invention provides a method of transfer of a gene of interest from a first vector to a product vector comprising generating a fused vector (the co-integrate vector, described hereinabove) comprising the first vector and a second vector, followed by rescue of the product vector from the fused vector by rolling circle replication. The Rep protein is involved in the rescue of the product vector from the fused vector by causing the initiation of rolling circle replication.

As described in Figure 2, the first step in replication by the rolling circle mechanism involves an incision being made by a protein termed Rep, at the double-stranded origin of replication or (+) origin of replication. Second, the 5' end of the incision site serves as the priming site for DNA synthesis, progressively replacing the strand with the covalently attached incising protein. When the replication fork reaches the double-stranded origin again, an incision is made in the displaced strand followed by circularization of the ends by ligation. The result is a relaxed, closed circular double-stranded DNA molecule containing the newly synthesized leading strand, and a single-stranded circular molecule consisting of the displaced strand. The nick is then sealed by the host cell DNA ligase, and the double-stranded DNA is then supercoiled by DNA gyrase. In a third step, DNA synthesis is initiated at a site on the single-stranded molecule referred to as the single-stranded origin of replication, or (-) origin of replication, thus converting the single-stranded plasmid into a double-stranded form. Finally, the DNA ends are joined by DNA ligase, and the resultant double-stranded DNA is supercoiled by DNA gyrase.

As a consequence, any sequence located between two double-stranded origins of replication can be converted into a circular plasmid in a host strain providing the incising protein described above, providing a single-stranded origin or replication is present on the (+) strand (Komberg and Baker (1992) DNA Replication 2.sup.nd Ed., Freeman and Company, NY; del Solar et al. (1993) Mol. Microbiol. 8:789; Khan (1997) Microbiol. Mol. Biol. Rev. 61:442).

In view of the instant claim amendments and remarks, Applicant respectfully requests reconsideration and withdrawal of the rejections.

Allowable Claims

Applicant acknowledges with appreciation the statement in the Office action that claim 11 is allowable.

Conclusion

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

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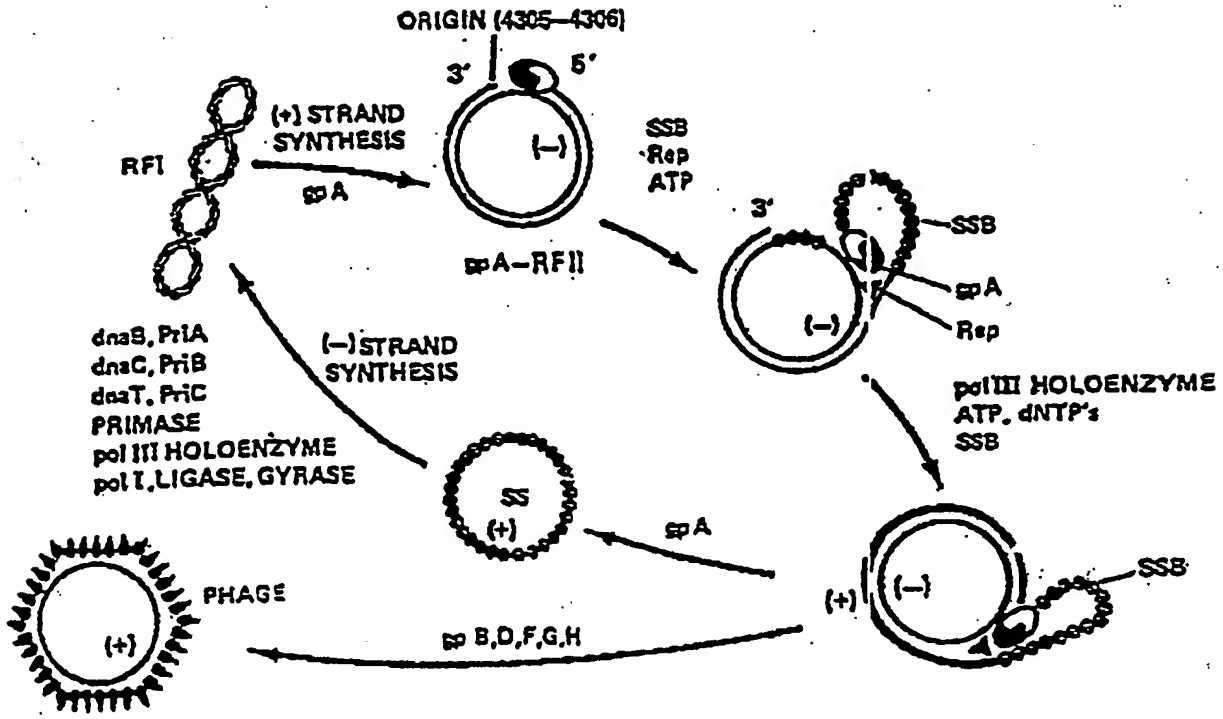
ANNOTATED

Figure 2



Prior Art ← ADDED